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DRAFT GUIDANCE DOCUMENT ON ACUTE INHALATION TOXICITY TESTING

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INTRODUCTION

1. Acute inhalation toxicity is the total of adverse effects caused by a substance following a single, uninterrupted exposure by inhalation over a short period of time to an airborne substance. For testing a fixed duration of exposure of 4 hours is generally recommended. Determination of the acute inhalation toxicity is usually an initial step in the assessment and evaluation of the toxic characteristics of a substance that may be inhaled such as a gas, volatile substance, or aerosol/particle (dusts and mists). It provides information on health hazards likely to arise from short-term exposure by the inhalation route. An evaluation of acute toxicity data should include the relationship, if any, between the animals' exposure to the test substance and the incidence and severity of all abnormalities, including behavioural and clinical abnormalities, the reversibility of observed abnormalities, gross lesions, body weight changes, effects on mortality, and any other toxic effects. If the particle size distributions of solid test substances or application aerosols show the presence of only minimal amounts of particles of toxicological relevance, other exposure routes should be considered more relevant for the determination of acute toxicity. Data from an acute study may serve as a basis for classification and labelling. In this context it is important to consider that for inhalation testing elaborate technical measures are taken to maximise the intensity of exposure and respirability of particles. Therefore, classification and labelling should take into account the fraction of particles of toxicological relevance present in the substance under conditions of normal handling and use.

2. For definitions see Annex I.

Particle-size distribution

3. The particle-size distribution of aerosolised test substances should allow exposure of all relevant regions of the respiratory tract of the tested animals. Particle-size also influences the site of predominant deposition in the respiratory tract. Damage and/or deposition to any region of the respiratory tract can induce lethality. It is not possible to predict, *a priori*, the most responsive region of the tract or the most harmful particle-size range that deposits throughout the entire rodent respiratory tract. An aerosol bracketing a particle-size mass distribution of the mass median aerodynamic diameter (MMAD) of 1 to 4 µm therefore appears to be most appropriate (1). For hygroscopic aerosols it should be taken into account that the size of the particles will increase as a result of water uptake. The Geometric Standard Deviation (GSD) should ideally be in the range of 1.5 to 3.0. Expert judgement should be provided if these range cannot be met.

Limit test

4. In the limit test, a single group of five males and five females is exposed to 5 mg/L of aerosol (solid, liquid) or 20 mg/L of a gas or vapour for 4 h, or where this is not possible due to physical or chemical properties of the test substance, the maximum attainable concentration, using the procedures described in this Guidance Document. Clear justification has to be provided if the respective limit concentration cannot be attained. If the objective of the test is solely related to hazard classification, the Acute Toxic Class method (ATC) should be given preference using three animals per group and sex. Details of the ATC-method are described in detail elsewhere (2)(3)(4)(5)(6)(7). Where necessary, a suitable non-toxic vehicle may be added to the test substance to help generate an appropriate concentration and respirability of the test substance in the atmosphere. Where a vehicle is used to attain the desired concentration of the substance in the atmosphere the acute inhalation toxicity of the vehicle used should be known. A concurrent vehicle (or any other) control group is not considered to be necessary if adequate historical data are available. Particulate material may be subjected to mechanical processes to decrease the particle-size. If no lethality is demonstrated up to the limit concentration, no further testing for acute

inhalation toxicity is needed. If substance-related mortality is produced, further study may need to be considered.

The acute toxic class (ATC) method

5. The ATC method (2) is a stepwise procedure with the use of three animals of each sex per step (2). Depending on the mortality and/or the moribund status of the animals, on the average 2-4 steps may be necessary to allow a judgement on the acute toxicity of the test substance. This procedure results in the use of substantially fewer animals than the method described in this Guidance document for the determination of the LC_{50} (8) while allowing for acceptable data-based scientific conclusions. The ATC method is based on biometric evaluations and the biometric model used is identical for both the oral (9)(TG 403) and the inhalation ATC method (7).

General principles

6. For acute inhalation toxicity studies the preferred mode of exposure is the nose-only, head-only or head/nose-only exposure technique. This mode of exposure minimises exposure or uptake by non-inhalation routes. By minimisation of the dermal contamination, the potential exposure of technical staff to as yet unknown test substances while handling the animals is also minimised. Additionally, this mode of exposure allows testing of high concentrations as required for limit tests and also reduces the consumption of test material. Also the instability of test substances (*e.g.,* reactivity with excreta or humidity) or the possible heterogeneity of the test atmosphere in this type of inhalation chambers is of limited concern. The duration required to attain the inhalation chamber equilibration is minimal. Such chambers are commercially available and relatively flexible to test low to high concentrations of vapours or aerosols (liquid or solid). However, there is also an option of using other systems (*e.g.,* whole-body inhalation chambers) when justification can be provided. Principles of the head-nose only and whole-body exposure techniques and their particular advantages and disadvantages are published in detail (10).

EXPOSURE OF ANIMALS AND PRINCIPLE OF TEST METHOD

Animals

7. Healthy young adult animals are acclimatised to the laboratory conditions for at least 5 days prior to the test. Before the test, animals are randomised and assigned to the required number of groups. Although several mammalian test species may be used, the preferred species is the rat. Commonly used laboratory strains should be employed. If another mammalian species is used, the tester should provide justification and reasoning for its selection. Young adult rats between 8–12 weeks old at the beginning of the study, should be used. The weight variation in animals or between groups used in a test should not exceed ± 20 percent of the mean weight of each sex. Several groups of experimental animals are exposed to the test substance in graduated concentrations for a defined period, one concentration being used per group. Subsequently, observations of effects and death are made. Animals that die during the test are necropsied and at the conclusion of the test surviving animals are sacrificed and necropsied. This Guidance Document is directed primarily to studies in rodent species but may be adapted for studies in non-rodents. Animals showing severe and enduring signs of distress and pain or evident signs of toxicity should be humanely killed and considered as animals that died on test. Exposure of experimental animals to test substances in a way known to cause marked pain and distress due to corrosive or irritating properties should not be performed.

Numbers and sex

8. In conventional tests for LC_{50} determinations, at least five experimentally naive animals are used at each concentration. The use of fewer animals may be justified (three animals per group and sex) when the ATC method is used. Females should be nulliparous and non-pregnant. Where adequate information is available to demonstrate that animals of one sex are markedly more sensitive, testing of the less sensitive sex is not required. In acute toxicity tests with animals of a higher order than rodents, the use of smaller numbers should be considered.

Housing

9. Each animal should be assigned a unique identification number. A system to assign animals to test groups and control groups randomly is required. The animals may be group-caged by sex, but the number of animals per cage should not interfere with clear observation of each animal. The biological properties of the test substance or toxic effects (*e.g.*, morbidity, excitability) may indicate a need for individual caging when the exposure mode is whole-body. Animals should be housed individually in whole-body inhalation chambers during exposure to aerosols.

Test conditions

10. The animals should be tested with inhalation equipment designed to sustain an adequate dynamic air flow, oxygen content of at least 19%, and uniform conditions throughout the exposure chamber. Description of exposure apparatus including source, design, type, dimensions, conditioning of air (supplied into and extracted from the chamber), system for the generation and characterisation of the test atmosphere, description of sampling conditions used for the characterisation of test atmosphere. Source and description of equipment used for calibrating and measuring temperature, humidity, air flows and volumes, and metering devices. Description of the determination and evaluation of vapour and/or aerosol concentrations with the corresponding particle-size distribution should be given.

Exposure concentrations

11. Variability in test concentration should be minimized to assure exposure at a steady-state inhalation chamber concentration. In the case of potentially explosive or inflammable test substances, care should be taken to avoid generating explosive concentrations. To establish suitable exposure concentrations a technical trial test without animals is recommended.

Nose-only exposure technique

12. During exposure, the animals are exposed to the test substance in exposure tubes. The animal restraining tubes should not impose undue stress on the animal. Animal restraining tubes should be constructed in such a way to avoid thermal stress. Urine and faeces should escape from the restrainer during the course of exposure. To provide optimal exposure of animals a slight positive balance of air volumes supplied to and extracted from the exposure system should be ensured. The design of the restraining tube as well as the flow dynamics should make it impossible for the subject to avoid inhalation exposure. When a negative balance of air volumes supplied and extracted is used a dilution of test atmosphere by bias-airflow (via exposure tubes) should be prevented. The inhalation chamber should be operated in well ventilated chemical hoods. Maintenance of slight negative pressure inside the hood will prevent leakage of the test substance into the surrounding areas. The animals should be exposed in inhalation equipment designed to sustain a dynamic air flow insuring an adequate air exchange (past flow exposure systems > 15/min, directed flow exposure systems at least 2 times the minute volume of all animals exposed). During the collection of test atmosphere a significant disturbance of the airflow

dynamics should be avoided. The rate of air flow should be adjusted to ensure that conditions throughout the equipment are essentially the same and temporally stable throughout the course of exposure.

Whole-body exposure technique

13. The animals should be tested with inhalation equipment designed to sustain a dynamic air flow of at least 10 air changes per hour. Other air flow rate may be useful to meet specific requirements imposed by the test substance. The chamber design should minimize crowding of the test animals and maximize their exposure to the test substance. To ensure stability of a chamber atmosphere, the total volume of the test animals should not exceed 5% of the volume of the test chamber. Maintenance of slight negative pressure inside the chamber will prevent leakage of the test substance into the surrounding areas.

Sampling of chamber atmosphere

14. A dynamic inhalation system with a suitable control system is desirable to monitor the inhalation chamber temperature and humidity. The samples should be taken from the vicinity of the breathing zone of the test species. During sampling the air flow should be monitored in regular intervals to detect changes in flow rate caused by an increased resistance in the adsorbent used. If impingers or gas bubblers containing volatile liquids (except water) are used during sampling of test atmosphere, evaporation of the solvent should be taken into account. Sampling ports should be designed in such a way that potential sampling errors as a result of anisokinetic sampling or by size-selective sampling are minimised. The tolerance limits for the radius of the sample probes are calculated according to published formulas (11)(12)(13). Further details are provided in handbooks of aerosol physics. These considerations are of relevance when the different devices used in one study provide inconsistent results of measurements.

Characterisation of test atmosphere

15. Ideally, the temperature in the chamber should be maintained at 22 ± 2 ºC, the relative humidity should be maintained between 30 % and 70 %, if practical. The method of generating and using water as vehicle or the aerosolization of high concentrations of dry particulate matter may preclude this condition. The consistency of the concentration of the substance in the test atmosphere should be monitored at regular intervals. Ideally, a monitoring device (*e.g.,* aerosol photometer for particulates or a total hydrocarbon analyser for volatile materials) may be used to demonstrate that temporally stable exposure conditions prevailed and that the time required to reach the inhalation chamber equilibrium concentration is negligible in relation to the total duration of exposure or is adequately taken into account. It should be noticed that monitoring of the test atmosphere is an integral measurement of all dynamic parameters and hence provides an indirect, however, integrative means to control all relevant, dynamic inhalation parameters (14). Therefore, the frequency of measurement of air flows (*vide infra*) may be reduced to one single measurement per exposure day. However, instruments may not be suitably used when their sensing units get covered with excessive quantities test material. Therefore, for high concentrations of particulate materials, an assessment should be made whether the monitoring of physical chamber parameters generate relevant data. If necessary, the rate of air flows should be adjusted regularly to ensure that all relevant conditions throughout the equipment are essentially the same. The characterization of test atmosphere should be representative for that atmosphere being inhaled by the test species.

Exposure time

16. The duration of exposure should be at least 4 hours after equilibration of the chamber concentration. Other durations may be needed to meet specific requirements.

Observation period

17. The observation period should be at least 14 days. However, the duration of observation should not be fixed rigidly. It should be determined by the toxic reactions, rate of onset, and length of recovery period, and thus should be extended when considered necessary. The time at which signs of toxicity appear, their duration, and the time of death are important, especially if there is a tendency for deaths to be delayed.

Observation of animals

18. A careful clinical examination should be made at least once each day. Additional observations should be made daily with appropriate actions taken to minimize loss of animals to the study, e.g., necropsy or refrigeration of those animals found dead and isolation of weak or moribund animals. Observations should be detailed and carefully recorded. Observations should include, but not be limited to, evaluation of skin and fur, eyes and mucous membranes, respiratory and circulatory effects, autonomic effects such as salivation, central nervous system effects, including tremors and convulsions, changes in the level of activity, gait and posture, reactivity to handling or sensory stimuli, altered strength, and stereotypes or bizarre behaviour (*e.g.,* self mutilation, walking backwards). Individual weights of animals should be determined prior to exposure, weekly after exposure, and at death. Changes in weights should be calculated and recorded when survival exceeds 1 day. The time of death should be recorded as precisely as possible.

Gross pathology

19. At the end of the test, surviving animals should be weighed and sacrificed. A gross necropsy should be performed on all animals under test, with particular reference to any changes in the respiratory tract. All gross pathology changes should be recorded. If necropsy cannot be performed immediately after a dead animal is discovered, the animal should be refrigerated (not frozen) at temperatures low enough to minimize autolysis. Necropsies should be performed as soon as possible, normally within a day or two.

Additional evaluations

20. In animals surviving 24 h or more, microscopic examination of organs showing evidence of gross pathology should be considered since it may yield useful information.

Test Atmosphere Characterisation

21. For the characterisation of test atmospheres the following measurements/calculations should be considered:

Characterisation of test atmospheres - volatile liquids

- Calculation of nominal concentrations.
- Determination of the analytical concentration during the course of exposure. Frequency: at least 3-times/exposure period.

Characterisation of test atmospheres - non-volatile liquids

- Calculation of nominal concentrations.
- Discussion of procedures used to improve the respirability of particles and to generate high test concentrations.
- Determination of the analytical concentration during the course of exposure. Frequency: at least 3-times/exposure period.
- Recovery of test substance (nominal versus analytical concentration)
    i. Characterisation of particle distribution. Frequency: once/exposure period

Substances of moderate vapour pressure may form an atmosphere consisting of an equilibrium of aerosol and vapour phase. Determination of the analytical concentration should take into account the overall concentration.

Characterisation of test atmospheres - powder, solid materials

-Determination of the analytical concentration during the course of exposure. Frequency: at least 3-times/exposure period. For solid non-volatile test substances (powders and liquids) gravimetric and chemical-analytical procedures are considered to be equivalent, if the preparation in air is analogous to the preparation prior to the test.

- Recovery of test substance (nominal versus analytical concentration)

-Characterisation of particle-size distribution. Frequency: at least once/exposure period (depending on the stability of atmosphere).

22. Whenever the test material is a mixture, the analytical procedure should be designed to evaluate the concentration of the total preparation. The concentration measurements may be based on analyses of specific ingredients of the mixture results of which have to be converted to represent the concentration of the total preparation. If there is some difficulty in measuring chamber analytical concentration due to precipitation, non homogeneous mixtures, volatile components, or other factors, additional analyses of inert components may be necessary.

Test report

23. In regard to the description of technical aspects the test report should include information on:

- test substance;
- vehicle;
- test animals;
- test conditions;
- the inhalation chamber;
- characterisation of the test atmosphere, and;
- exposure data.

As described in the ATC Test Guideline.

Discussion of results and relevance of results for labelling and classification

24. The possible causal relationship of exposure and lethality should be established. Emphasis should be caused by local effects within the respiratory tract the exposure atmosphere or particle-size generated during the test may not necessarily be relevant for atmospheres of particulate materials likely to occur under actual conditions of production or use of the substance. If respective data of the test substance would demonstrate that the conditions used in this guidance document can be no means be met under real-life exposure conditions, labelling and classification should be made using the criteria given in Annex 2.

ANNEX I

DEFINITIONS

Acute inhalation toxicity is the total of adverse effects caused by a substance following a single uninterrupted exposure by inhalation over a short period of time to an airborne substance.

Aerodynamic diameter applies to the behavioural size of particles of aerosols. It is the diameter of a sphere unit density which behaves aerodynamically as the particle of the test substance. It is used to compare particles of different sizes, shapes, and densities to predict where in the respiratory tract such particles may be deposited. This term is used in contrast to "optical", "measured", or "geometric diameter" which is a representation of actual diameters which in themselves cannot be related to deposition within the respiratory tract.

Aerosol: A suspension of solid or liquid particles in a suspension in a gas, as a foam, paste or powder or in a liquid state or in a gaseous state.

Analytical or actual concentrations refer to concentrations obtained by sampling of test atmosphere in that location of an inhalation chamber which is being inhaled by the test species investigated. Hazard assessment can only be performed on the basis of analytical or 'actual' exposure concentrations. Nominal concentrations are less relevant for hazard assessment since it depends heavily on specific procedures and may differ from one laboratory to another. Reactive test substances may decompose in humid chamber atmospheres which is only addressed adequately by analytical concentrations.

Concentration is expressed as weight of the test substance per unit volume of air, for vapours and dusts as mg/L and for gases as ppm (parts per million), in accordance with the UN GHS.

Exposure concentration is the actual concentration of test substance the test animal is exposed to. It is determined by the analytical characterisation of the test atmosphere in the vicinity of the breathing zone of the animals exposed. It is commonly expressed in mass (mg) per unit volume (L) of air. The mass of test substance per unit mass of test animal (e.g., mg/kg) which is equal the dose, is difficult to define in inhalation toxicity since the fraction of substance absorbed/retained in the respiratory tract or absorbed via the gastrointestinal tract is dependent on a number of variable often not defined of measured in acute inhalation studies. Due to these uncertainties, exposure should be defined in terms of "exposure concentrations" rather than "exposure doses".

Geometric standard deviation (GSD) (see Mass Median Aerodynamic Diameter (MMAD).

GHS - Globally Harmonized System of Classification and Labelling of Chemicals: a system proposing the classification of chemicals according to standardised types and levels of physical, health and environmental hazards, and addressing corresponding communication elements, such as pictograms, signal words, hazard statements, precautionary statements and safety data sheets, so that to convey information on their adverse effects with a view to protect people and the environment. A joint activity of OECD (human health and the environment), UN Committee of Experts on Transport of Dangerous Goods (physical-chemical properties) and ILO (hazard communication) and co-ordinated by the Interorganisation Programme for the Sound Management of Chemicals (IOMC) (15).

Inhalable diameter refers to that aerodynamic diameter of a particle which is considered to be inhalable for the test species. It is used to refer to particles which are capable of being inhaled and deposited anywhere within the respiratory tract. The exact definitions are as follows:

a) ***Inspirable Particulate Mass*** for those materials which are hazardous when deposited anywhere in the respiratory tract.

b) ***Thoracic Particulate Mass*** for those materials which are hazardous when deposited anywhere within the lung airways and the gas-exchange region.

c) ***Respirable Particulate Mass*** for those materials which are hazardous when deposited in the gas exchange region.

LC_{50} (median lethal concentration) is a statistically derived estimate of a concentration of a substance that can be expected to cause death during exposure or within a fixed time after exposure in 50 percent of animals exposed for a specified time. The LC_{50} value is expressed as weight of test substance per unit volume of air (mg/L). For gases parts per million may also be used. For clarity, the exposure duration should also be specified, *e.g.*, 4–h LC_{50}.

Mass Median Aerodynamic Diameter (MMAD) is the calculated aerodynamic diameter which devides the particles of an aerosol in half based on mass of the particles. Fifty per cent of the particles by mass will be larger than the median diameter and fifty per cent of the particles will be smaller than the median diameter. The median diameter and its **geometric standard deviation (GSD)** is used to statistically describe the particle-size distribution of any aerosol (liquid or solid) based on the mass size of the particles.

Moribund status: being in a state of dying or inability to survive, even if treated. (See the Humane Endpoint Guidance Document (16) for more details).

Nominal concentration. The nominal concentration was calculated from the ratio of the total quantity of test substance consumed during the exposure period and the total throughput of air through the inhalation chamber using the formula shown below.

$$Nom.Concentration[mg/m^3] = \frac{\left(\Sigma\, mass\, consumed \times conc.of\ \ solution[w/v]\right)}{flow\, rate[m^3/\min] \times time[\min]}$$

Vapour: The gaseous form of a substance or mixture which is normally in liquid or solid state at ambient conditions of temperature and pressure.

ANNEX II

CALCULATION OF MMAD AND GSD

Particle Size

1. Samples for the analysis of the particle size distribution are from an inhalation chamber location which is representative for the exposure zone of the subject under investigation. The particle-size distribution should be analysed using impaction devices measuring the mass related aerodynamic diameter. If applicable, each individual impactor stage should be covered with an adhesive coating to prevent particle bouncing. By using appropriate assay methods, the mass-related aerodynamic particle-size distribution of the test substance can conveniently be determined. In addition to the size distribution, a material balance may be obtained by comparison with the determination of the total concentration of test substance in the inhalation chamber. Other methods may be used to overcome the long sampling periods required for cascade impactors or when evaporation losses are likely to occur within the impactor.

Calculation of the Mass Median Aerodynamic Diameter (MMAD) of the aerosol collected in the cascade impactor

2. Construct a 'Cumulative Percent Found - Less Than Stated Particle Size' table, calculate the total mass of test substance collected in the cascade impactor. Start with the test substance collected on the stage that captures the smallest particle-size fraction, and divide this mass of the test substance by the total mass found above. Multiply this quotient by 100 to convert to percent. Enter this percent opposite the effective cut-off diameter of the stage above it in the impactor stack. Repeat this step for each of the remaining stages in ascending order. For each stage, add the percentage of mass found to the percentage of mass of the stages below it. Plot the percentage of mass less than the stated size versus particle size in a probability scale against a log particle-size scale, and draw a straight line best fitting the plotted points (Figure 1). If necessary, a weighted least square regression analysis may be used to achieve the best fit. Note the particle size at which the line crosses the 50% mark. This is the estimated MMAD.

Calculation of Geometric Standard Deviation (GSD)

3. Refer to the log probability graph used to calculate the MMAD. Provided that the line is a good fit to the data, the size distribution is log normal, and the calculation of the GSD is appropriate. Note that particle size at which the line crosses the 84.1% mark. Note the particle size at which the line crosses the 15.9% mark. Calculate the GSD by the following equation(s):

$$GSD = \frac{84.1\%\,mark}{50\%\,mark} \text{ or } GSD = \frac{50\%\,mark}{15.9\%\,mark}$$

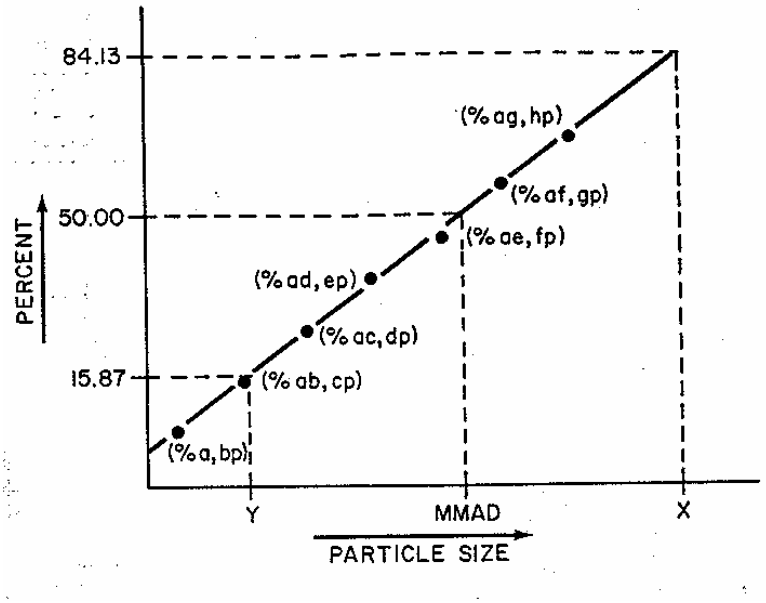


Figure 1: Plot of the percentage of mass less than the stated size (probability scale) *versus* aerodynamic particle size (log scale).

4. To verify graphically that the aerosol is in fact unimodal and log-normally distributed the normalized mass per stage (f_H') is evaluated as a histogram. $\Delta\log D_p$ is equal the difference $\log D_{p+1}$ - $\log D_p$, whereas D_p is the lower cut-size limit and D_{p+1} the higher cut-size limit of the corresponding impactor stage. Calculate the histogram f_H' by equation:

$$f_H^{'} = N_f \times \frac{mass\ /\ stage}{\Delta \log D_p} \qquad (1)$$

Calculate the log-normal mass distribution $y'(D_{ae}) = N_f \times y(D_{ae})$ as a function of the aerodynamic diameter (D_{ae}) using by equation:

$$y^{'}(D_{ae}) = \exp\left[-\frac{(\log D_{ae} - \log MMAD)^2}{2 \times \log^2 GSD}\right] \qquad (2)$$

and use the normalization factor (N_f):

$$N_f = \left(\frac{\Sigma mass}{\log GSD \times \sqrt{2\pi}}\right)^{-1} \qquad (3)$$

5. An example calculation with corresponding table 1 and fig. 2 is provided as follows. It should be noted that this size distributions shown in Fig. 2 as well as in Annex 2 were constructed utilising equation 2.

Table 1: Cascade Impactor Analysis - Sample Calculation

N	Impactor stage (µm - µm)	Cut-off diameter (µm)	Mass/ stage (mg)	Relative mass (%)	Cumulative mass (%)
1	0.01 - 0.40	0.010	0.100	0.27	0.00
2	0.40 - 0.70	0.400	0.700	1.92	0.27
3	0.70 - 1.10	0.700	2.900	7.97	2.20
4	1.10 - 2.10	1.100	6.200	17.03	10.16
5	2.10 - 3.30	2.100	7.800	21.43	27.20
6	3.30 - 4.70	3.300	9.100	25.00	48.63
7	4.70 - 5.80	4.700	5.000	13.74	73.63
8	5.80 - 9.00.	5.800	3.800	10.44	87.36
9	9.00 - 30.00	9.000	0.800	2.20	97.80

Mass Median Aerodynamic Diameter (MMAD): 2.82 µm

Geometric standard deviation (GSD): 1.93

Number Median Aerodynamic Diameter (NMAD): 0.77 µm

Surface Median Aerodynamic Diameter (SMAD): 1.83 µm

System: X-IMPACTOR

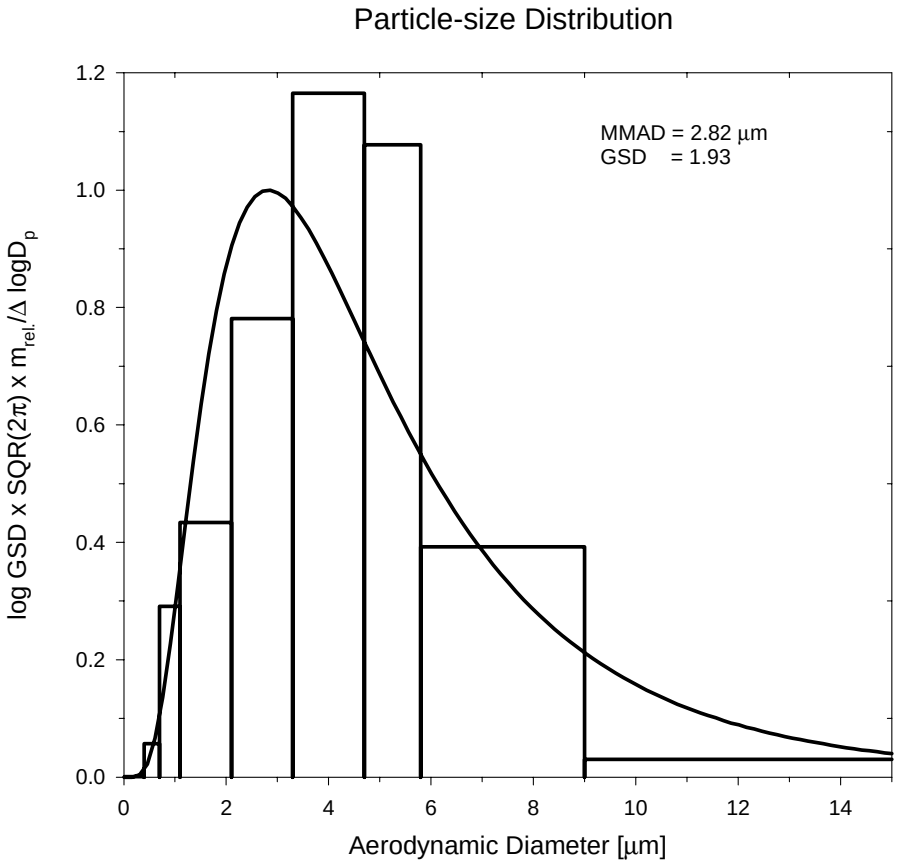
Air flow 4.00 L/min

Sampling time: 300.00 seconds

Concentration (computed): 1820.00 mg/m^3 air

Respirability percent < 3.0 µm: 53.7 %

Figure 2: Particle-size distribution h and histogram and log-normal distribution (equat. 2)



ANNEX III

LITERATURE

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